Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/006626

International filing date: 28 February 2005 (28.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/548,341
Filing date: 27 February 2004 (27.02.2004)

Date of receipt at the International Bureau: 09 May 2005 (09.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

1314600

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 27, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/548,341*
FILING DATE: *February 27, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/06626*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (08-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL983652713US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Arun Kumar	DHAR	San Diego, California

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

DISEASE CONTROL IN SHRIMP

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[x] Customer Number: 25225

OR

[ ] Firm or Individual Name					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS (*check all that apply*)

[x] Specification *Number of Pages*	250	[ ] CD(s), Number	
[x] Drawing(s) *Number of Sheets*	2	[x] Other (specify):	Return postcard
[x] Application Data Sheet. See 37 CFR 1.76 (2 pages)			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[x] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees.

[x] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1952

[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[x] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE

TYPED OR PRINTED NAME: Laurie L. Hill, Ph.D.

TELEPHONE: (858) 720-7955

Date: February 27, 2004

REGISTRATION NO. *(if appropriate)*: 51,804

Docket Number: 475443000800

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EL983652713US, in an envelope addressed to: MS Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: 2-27-04 Signature: _____ (Michael Boyd)

DISEASE CONTROL IN SHRIMP

Technical Field

[0001] The invention pertains to the identification, monitoring, and treatment of infection in crustaceans, particularly shrimp. The compositions and methods use nucleic acids and polypeptides identified by differentially expression in non-infected and infected crustaceans as therapeutics, diagnostics, and screening reagents.

Background of the Invention

[0002] During the past decade, shrimp (*Penaeus* sp.) farming has evolved from subsistence level farming to a major worldwide industry providing jobs to millions of people. Currently, shrimp farming remains a major source for economic development in the poor coastal areas of many countries in the Asia and the Americas (Rosenberry, 2002). As shrimp farming evolves, it is faces many challenges. Among these challenges, viral diseases are of major concern to shrimp farmers. More than 20 viruses have been reported to infect shrimp (Lightner 1996), and the list is growing. Many of these viruses have already caused serious disease in cultured and wild penaeid shrimp resulting in significant economic losses to commercial shrimp farmers. At present, white spot disease of shrimp, caused by the white spot syndrome virus (WSSV), is considered to be the most important viral disease of cultured shrimp worldwide (Office International de Epizooties, 2002). Since the initial report of WSSV in East Asia during 1992 to 1993 (Inouye *et al.* 1994), WSSV has spread to much of Asia and the Americas causing catastrophic losses to shrimp farmers (Krishna *et al.* 1997; Jory and Dixon 1999). The cumulative loss due to WSSV in the Asia since 1992 is estimated to be $4-6 billion. The losses due to WSSV in the Americas have been estimated to $1-2 billion.

[0003] WSSV virions are ellipsoid to bacilliform in shape, enveloped with a tail-like appendage at one end of the particle. The genome of WSSV contains a circular double-stranded DNA of ~300 kb in length (van Hulten *et al.* 2001; Yang *et al.* 2001). Although the WSSV has a morphological similarity with baculovirus, sequence analysis revealed that WSSV shares very little similarity with any known viruses (van Hulten et al, 2001; Yang *et*

al., 2001). As a result, WSSV has been placed in a new family, the *Nimaviridae*, and a new genus, *Whispovirus*.

[0004] WSSV infects all commercially important species of penaeid shrimp and a number of other crustaceans, including crabs and crayfish (Flegel 1997). Since the initial report of WSSV in East Asia during 1992 to 1993 (Inouye *et al.* 1994), a number of WSSV-encoded genes, such as the capsid genes (van Hulten *et al.* 2000a; van Hulten *et al.* 2000b; Zhang *et al.* 2001; Chen *et al.* 2002; Marks *et al.* 2003), a ribonucleotide reductase gene (Tsai *et al.* 2000a), and the thymidine kinases (Tsai *et al.* 2000b) have been studied in detail. In addition, a highly sensitive detection method based on real-time PCR has been developed for detecting and quantifying WSSV (Dhar *et al.* 2001). However, insight into immune genes in shrimp involved in WSSV pathogenesis is limited and only just beginning to emerge. For example, Dhar and colleagues cloned and sequenced a lipopolysaccharide and β-1,3-glucan binding protein (LGBP) gene from *Penaeus stylirostris* shrimp that shows up-regulation during WSSV infection (Roux *et al.*, 2002). LGBP, a pattern recognition protein (PRP), is known to elicit the expression of prophenoloxidase (proPO) during bacterial and fungal infection (Soderhall and Cerenius, 1998). Although LGBP gene expression is upregulated in WSSV-infected animals, proPO gene expression is downregulated as the WSSV infection progresses, suggesting that WSSV infection regulates the activation and / or activity of the prophenoloxidase cascade in a novel way (Roux *et al.*, 2002). A syntenin-like protein (TE8) with a post-synaptic density protein (PDZ) domain has been isolated from *Penaeus monodon* shrimp and was upregulation during WSSV-infection (Bangrak *et al.*, 2002). It has been suggested that the shrimp syntenin-like protein may function as an adapter that couples the PDZ-binding protein to cell-to-cell signal transduction during WSSV pathogenesis (Bangrak *et al.*, 2002). Antiviral substances capable of binding to a variety of DNA and RNA viruses (Sindbis virus, vaccinia virus, vesicular somatitis virus, mengo virus, banzi virus and poliomyelitis virus) have been isolated from shrimp (*Penaeus setiferus*), although the genes representing these proteins have not yet been cloned (Pan *et al.*, 2000). These antiviral proteins probably represent a component of the innate immune response in shrimp (Pan *et al.*, 2000). All the above examples of the characterization of immune genes and associated proteins in shrimp revealed the candidate gene approaches used by different researchers. Although such

approaches may provide in depth information on a particular gene, they do not provide a holistic view of gene expression during viral or microbial pathogenesis.

[0005] To mitigate these challenges and to promote sustainable shrimp farming, there is an urgent need to develop therapeutics against white spot disease and viral diseases in shrimp in general. One of the limitations in developing therapeutics against viral diseases of shrimp is the lack of information on the cellular genes that might be involved in WSSV pathogenesis. This invention addresses this need by identifying cellular genes that play a critical role in viral, specifically WSSV, pathogenesis in shrimp, and viral pathogenesis in invertebrates in general. Several immune genes in shrimp have been isolated that showed differential expression between healthy and WSSV-infected shrimp. These genes could be used as potential targets for developing therapeutics against white spot disease and other viral, bacterial, and fungal diseases in shrimp.

Brief Summary of the Invention

[0006] This invention has identified cellular genes that play a critical role in viral, specifically white spot syndrome virus (WSSV), pathogenesis in shrimp, and viral pathogenesis in invertebrates in general. Several immune genes in shrimp have been isolated that showed differential expression between healthy and WSSV-infected shrimp. The nucleic acids comprising these genes, or fragments thereof, can used as potential targets for developing therapeutics against white spot disease and other viral, bacterial, and fungal diseases in shrimp. Accordingly, the invention provides methods for screening for compounds for treating or diagnosing WSSV using the nucleic acids of the invention or the polypeptides they encode.

[0007] The invention provides compositions comprising, or consisting of, sets of differentially expressed genes selected by their differential expression on viral infection as compared to healthy shrimp expression profiles. In one aspect, the viral infection is white spot syndrome virus, Taura syndrome virus, infectious hypodermal and hematopoietic virus, yellowhead virus or baculovirus penae.

[0008] In one aspect, the genes are selected from expressed sequence tag libraries designed by differential selection of genes that are up- or down-regulated during viral infection. In one aspect, the viral infection is white spot syndrome virus. The genes can the genes described herein, e.g., the nucleic acids as set forth in Table 2 and the appendices. In

one aspect, the viral infection is selected from white spot syndrome virus, Tarua syndrome virus, infectious hypodermal and hematopoietic virus, yellowhead virus and baculovirus penae. The genes can be selected from the shrimp expressed sequence tag differential library used for the treatment of disease. The disease can be selected from white spot syndrome virus, Tarua syndrome virus, infectious hypodermal and hematopoietic virus, yellowhead virus and baculovirus penae. The differential library can be produced from shrimp infected with WSSV, or, from shrimp infected with viruses selected from white spot syndrome virus, Tarua syndrome virus, infectious hypodermal and hematopoietic virus, yellowhead virus and baculovirus penae.

[0009] The invention also provides methods of determining the metabolic or disease state of shrimp using the compositions described herein, including the polypeptides of the invention and the nucleic acids of the invention.

[0010] The invention provides isolated or recombinant nucleic acids having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity, when compared and aligned for maximum correspondence, as measured using one any known sequence comparison algorithm, as discussed in detail below, or by visual inspection, to an exemplary nucleic acid of the invention, which include all nucleic acids sequences described herein.

[0011] The invention provides isolated or recombinant polypeptides having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity, when compared and aligned for maximum correspondence, as measured using one any known sequence comparison algorithm, as discussed in detail below, or by visual inspection, to an exemplary polypeptides of the invention, which include all polypeptides described herein, and include all polypeptides encoded by nucleic acids of the invention, which include all nucleic acids sequences described herein.

[0012] In alternative aspects, the invention provides nucleic acid and polypeptide sequences having substantial identity to an exemplary sequence of the invention over a region of at least about 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more residues.

[0013] It is an object of the invention to provide a number of expressed sequence tags (ESTs) isolated from shrimp tissue that specifically correspond to the onset of viral disease.

[0014] It is a further object of the invention to provide a number of expressed sequence tags (ESTs) isolated from *Penaeid* shrimp tissue that specifically correspond to the onset of viral disease.

[0015] It is an object of the invention to provide a number of expressed sequence tags (ESTs) that have been isolated from shrimp (*Penaeus vannamei)* tissue and specifically correspond to the onset of viral disease.

[0016] It is an object of the invention to provide a method of protecting shrimp from viral infection using recombinant protein or nucleic acids derived from the full-length gene or recombinant truncated protein or nucleic acids derived from the functional domain of the gene identified using the EST differential libraries of the instant invention.

[0017] It is an object of the invention to provide a method of protecting shrimp from WSSV infection or other viral infection using recombinant protein or nucleic acids derived from the full-length gene or recombinant truncated protein or nucleic acids derived from the functional domain of the gene.

[0018] It is an object of the invention to provide a feed or feed supplement which incorporates recombinant protein or nucleic acids identified using the EST differential libraries of the instant invention and providing same to the animal to inhibit the deleterious effects of the virus on the host.

[0019] It is an object of the invention to provide a treatment that incorporates recombinant protein or nucleic acids identified using the WSSV infected and healthy EST differential libraries of the instant invention and providing it to the animal to inhibit or suppress the deleterious effects of the WSSV on the host.

[0020] It is an object of the invention to provide a therapeutic, a feed, or feed supplement that incorporates recombinant protein or nucleic acids identified using the

WSSV infected and healthy EST differential libraries of the instant invention and providing it to the animal to inhibit or suppress the deleterious effect of the WSSV on the host.

[0021] It is an object of the invention to provide a method of treatment for white spot viral disease in shrimp based on the genes identified through differential expression libraries.

[0022] It is an object of the invention to provide a method of treatment wherein the method comprises the steps including the production of recombinant protein using bacterial, yeast, plant, and/or algal expression systems, then mixing the recombinant protein with feed and delivering the recombinant protein in sufficient quantity to prevent action of the virus on shrimp. This approach can also be applied to diseases in shrimp caused other viruses, bacteria, fungi, as well as in other aquaculture species including fish and shellfish suffering from fungal, bacterial, and viral diseases. In aquatic invertebrate species, like shrimp, with a primitive immune system (i.e., one that is not antibody driven) the invention provides a method for treatment of both acute and chronic diseases via delivery of therapeutic recombinant protein(s) and/or nucleic acids.

[0023] It is an objective of this invention to use ESTs differentially expressed on viral infection as a biopesticide.

[0024] It is an object of the invention to provide a method using the differentially expressed genes from the EST differential library as a diagnostic tool to evaluate shrimp both in aquaculture and in food processing.

[0025] It is an object of the invention to provide a useful resource for functional genomics study in shrimp using microarray analysis and single nucleotide polymorphisms (SNPs) and a resource for microsatellite sequence useful for developing shrimp genome map.

[0026] It is an object of the invention to provide a feed or feed supplement containing recombinant protein(s) or nucleic acid(s), derived from the expressed sequence tags from healthy and WSSV-infected libraries, that can inhibit or suppress the deleterious effect of other shrimp viruses including the Taura syndrome virus, yellow head virus, and infectious hypodermal and hematopoietic necrosis virus and WSSV (Lightner and Redman, 1998).

[0027] It is an object of the invention to provide a method of treatment for bacterial diseases of shrimp and other aquaculture species based on the genes identified through shrimp EST analysis.

[0028] Therefore, the invention provides both a composition that can be used for prevention of disease, a method of prevention of disease, and diagnostic tools for evaluation of the health of the animals affected by disease.

Brief Description of the Drawings

[0029] FIG. 1 is a graphical representation of Function Classes of genes isolated from hepatopancreas cDNA libraries of healthy and WSSV infected shrimp (*P. vannamei*) that showed similarities with the GenBank database entries; and

[0030] FIG. 2 is a graphical representation of the differential expression of expressed sequence tags (ESTs) in white spot syndrome virus (WSSV) infected shrimp (*Penaeus vannamei*) compared to healthy shrimp (*P. vannamei*). Bars above the X-axis indicate up-regulation, and bar below the X-axis indicates down-regulation of the gene in WSSV-infected compared to healthy shrimp.

Detailed Description of the Invention

Definitions

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, published patent applications and other publications and sequences from GenBank and other databases referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in patents, published patent applications and other publications and sequences from GenBank and other data bases that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

[0032] As used herein, "a" or "an" means "at least one" or "one or more."

[0033] A "coding sequence" is an in-frame sequence of codons that (in view of the genetic code) correspond to or encode a protein or peptide sequence. Two coding

sequences show similarity or homology to each other if the sequences or their complementary sequences encode the same or similar amino acids.

[0034] An "EST" or "expressed sequence tag" is a piece of nucleic acid that is found under specific conditions and is derived from a cDNA library. A unique DNA sequence derived from a cDNA library (therefore from a sequence which has been transcribed in some tissue or at some stage of development). The EST can be mapped, by a combination of genetic mapping procedures, to a unique locus in the genome and serves to identify that genetic locus.

[0035] "Shrimp" refer to any of the group of crustaceans that are commonly cultured for aquaculture or captured in the wild fisheries. The term "shrimp" includes shrimp eggs, shrimp larvae, shrimp post-larvae and adult shrimp. The term "shrimp" and "prawn" will be used interchangeably throughout the specification. Shrimp an be, but are not limited to *Penaeus* shrimp and include the species *Penaeus vannamei, Penaeus chinensis, Penaeus monodon, Penaeus stylirostris, Penaeus japonicus, Penaeus penicillatus, Penaeus merguiensis, Penaeus indicus, Penaeus subtilis, Penaeus paulensis, Penaeus setiferus, Penaeus brasiliensis, Penaeus duorarum, Penaeus occidentalis, Penaeus schmitti, Penaeus californiensis, Penaeus semisulcatus, Penaeus latisulcatus, Metapenaeus monoceros, Metapenaeus dobsoni, Metapenaeus affinis*, and *Metapenaeus brivicornis*; and Litopenaeid shrimp (such as *Litopenaeus vannamei, L. setiferus*).

[0036] "Contig" refers to a continuous sequence of DNA that has been assembled from overlapping nucleotide sequence of cDNA clones.

[0037] "Singleton" refers to a single EST sequence representing a particular gene.

[0038] "Unigene set" refers to non-redundent set of gene-oriented clusters.

[0039] The term "antibody" includes a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope, see, e.g. Fundamental Immunology, Third Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994) J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (*e.g.*, fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent

fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

[0040] The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface, as discussed in further detail, below.

[0041] The term "expression cassette" as used herein refers to a nucleotide sequence which is capable of affecting expression of a structural gene (i.e., a protein coding sequence, such as an recombinant protein encoded by a nucleic acid of the invention) in a host compatible with such sequences. Expression cassettes include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences, *e.g.*, transcription termination signals. Additional factors necessary or helpful in effecting expression may also be used, *e.g.*, enhancers. Thus, expression cassettes also include plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like.

[0042] "Operably linked" as used herein refers to a functional relationship between two or more nucleic acid (*e.g.*, DNA) segments. Typically, it refers to the functional relationship of transcriptional regulatory sequence to a transcribed sequence. For example, a promoter is operably linked to a coding sequence, such as a nucleic acid of the invention, if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are *cis*-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0043] A "vector" comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (*e.g.*, a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (*e.g.*, RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (*e.g.*, plasmids, viruses, and the like, see, *e.g.*, U.S. Patent No. 5,217,879), and include both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

[0044] As used herein, the term "promoter" includes all sequences capable of driving transcription of a coding sequence in a cell, *e.g.*, a plant cell. Thus, promoters used in the constructs of the invention include *cis*-acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene. For example, a promoter can be a *cis*-acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These cis-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, etc.) transcription. "Constitutive" promoters are those that drive expression continuously under most environmental conditions and states of development or cell differentiation. "Inducible" or "regulatable" promoters direct expression of the nucleic acid of the invention under the influence of environmental conditions or developmental conditions. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, drought, or the presence of light.

[0045] "Plasmids" can be commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. Equivalent plasmids to those described herein are known in the art and will be apparent to the ordinarily skilled artisan.

[0046] The term "gene" includes a nucleic acid sequence comprising a segment of DNA involved in producing a transcription product (*e.g.,* a message), which in turn is translated to produce a polypeptide chain, or regulates gene transcription, reproduction or stability. Genes can include regions preceding and following the coding region, such as leader and trailer, promoters and enhancers, as well as, where applicable, intervening sequences (introns) between individual coding segments (exons).

[0047] The phrases "nucleic acid" or "nucleic acid sequence" includes oligonucleotide, nucleotide, polynucleotide, or to a fragment of any of these, to DNA or RNA (*e.g.,* mRNA, rRNA, tRNA) of genomic or synthetic origin which may be single-stranded or double-stranded and may represent a sense or antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material, natural or synthetic in origin, including, *e.g.,* iRNA, ribonucleoproteins (*e.g.,* iRNPs). The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones, see *e.g.,* Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197; Strauss-Soukup (1997) Biochemistry 36:8692-8698; Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156.

[0048] "Amino acid" or "amino acid sequence" include an oligopeptide, peptide, polypeptide, or protein sequence, or to a fragment, portion, or subunit of any of these, and to naturally occurring or synthetic molecules. The terms "polypeptide" and "protein" include amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain modified amino acids other than the 20 gene-encoded amino acids. The term "polypeptide" also includes peptides and polypeptide fragments, motifs and the like. The term also includes glycosylated polypeptides. The peptides and polypeptides of the invention also include all "mimetic" and "peptidomimetic" forms, as described in further detail, below.

[0049] The term "isolated" includes a material removed from its original environment, *e.g.,* the natural environment if it is naturally occurring. For example, a naturally occurring

polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. As used herein, an isolated material or composition can also be a "purified" composition, i.e., it does not require absolute purity; rather, it is intended as a relative definition. Individual nucleic acids obtained from a library can be conventionally purified to electrophoretic homogeneity. In alternative aspects, the invention provides nucleic acids which have been purified from genomic DNA or from other sequences in a library or other environment by at least one, two, three, four, five or more orders of magnitude.

[0050] As used herein, the term "recombinant" can include nucleic acids adjacent to a "backbone" nucleic acid to which it is not adjacent in its natural environment. In one aspect, nucleic acids represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid "backbone molecules." "Backbone molecules" according to the invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. In one aspect, the (isolated, recombinant, enriched) nucleic acids represent 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. "Recombinant" polypeptides or proteins refer to polypeptides or proteins produced by recombinant DNA techniques; *e.g.*, produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide or protein. "Synthetic" polypeptides or protein are those prepared by chemical synthesis, as described in further detail, below.

[0051] A promoter sequence can be "operably linked to" a coding sequence when RNA polymerase which initiates transcription at the promoter will transcribe the coding sequence into mRNA, as discussed further, below.

[0052] "Oligonucleotide" includes either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide can ligate to a fragment that has not been dephosphorylated.

[0053] The phrase "substantially identical" in the context of two nucleic acids or polypeptides, can refer to two or more sequences that have, *e.g.,* at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more nucleotide or amino acid residue (sequence) identity, when compared and aligned for maximum correspondence, as measured using one any known sequence comparison algorithm, as discussed in detail below, or by visual inspection. In alternative aspects, the invention provides nucleic acid and polypeptide sequences having substantial identity to an exemplary sequence of the invention over a region of at least about 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more residues, or a region ranging from between about 50 residues to the full length of the nucleic acid or polypeptide. Nucleic acid sequences of the invention can be substantially identical over the entire length of a polypeptide coding region.

[0054] A "substantially identical" amino acid sequence also can include a sequence that differs from a reference sequence by one or more conservative or non-conservative amino acid substitutions, deletions, or insertions, particularly when such a substitution occurs at a site that is not the active site of the molecule, and provided that the polypeptide essentially retains its functional properties. A conservative amino acid substitution, for example, substitutes one amino acid for another of the same class (*e.g.,* substitution of one hydrophobic amino acid, such as isoleucine, valine, leucine, or methionine, for another, or substitution of one polar amino acid for another, such as substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine). One or more amino acids can be deleted, for example, from a recombinant protein encoded by a nucleic acid of the invention, resulting in modification of the structure of the polypeptide, without significantly

altering its biological activity. For example, amino- or carboxyl-terminal amino acids that are not required for the protein's activity can be removed.

[0055] "Hybridization" includes the process by which a nucleic acid strand joins with a complementary strand through base pairing. Hybridization reactions can be sensitive and selective so that a particular sequence of interest can be identified even in samples in which it is present at low concentrations. Stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. For example, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature, altering the time of hybridization, as described in detail, below. In alternative aspects, nucleic acids of the invention are defined by their ability to hybridize under various stringency conditions (*e.g.,* high, medium, and low), as set forth herein.

[0056] "Variant" includes polynucleotides or polypeptides of the invention modified at one or more base pairs, codons, introns, exons, or amino acid residues (respectively) yet still retain the biological activity of the recombinant polypeptide of the invention.

ESTs, nucleic acids, polypeptides, and fragments thereof

[0057] Provided herein is a composition consisting of a set of differentially expressed gene selected by their differential expression in viral-infected shrimp relative to non-virally-infected, or healthy shrimp. Expressed sequence tag (EST) analysis is an effective, comprehensive, and relatively straightforward method of examining gene expression. This method is particularly useful for species where no or limited information is available regarding the genome of the species. An EST approach was used to examine gene expression in different tissues (cephalothorax, eyestalks, and pleopod) in black tiger shrimp, *P. monodon* (Lehnert *et al.*, 1999). The putative identities of many of these ESTs revealed the occurrence of tissue-specific expression that includes novel genes. A number of immune genes have also been isolated from the hemocyte and hepatopancreas cDNA libraries from cultured specific pathogen free (SPF) *Penaeus (Litopenaeus) vannamei* shrimp and wild *P. setiferus* (Gross *et al.*, 2001). Among these shrimp, anti-microbial peptides and lectins were most prevalent in the hemocyte and hepatopancreas cDNA libraries, respectively (Gross *et al.*, 2001). Immune genes isolated by EST analysis from a

hemocyte cDNA library of *P. monodon* include genes that are involved in the clotting system and the prophenoloxidase-activating system, as well as antioxidative enzymes, antimicrobial peptides, and serine protease inhibitors (Supungul *et al.*, 2002). Recently, Rojtinnakorn *et al.* (2002) compared the mRNA expression profiles of healthy and WSSV-infected kuruma prawns (*Penaeus japonicus*) by EST analysis of hemocytes. Hemocytes and hepatopancreas tissue represent the core of the primitive immune system found in shrimp and most other invertebrates. Humoral immune responses are initiated in the shrimp hepatopancreas while both humoral and cellular components of the immune system are found in the hemocyte population. Applicants are unaware of any prior analysis of hematopancreatic gene expression in the presence or absence of viral infection in shrimp.

[0058] Thus, one aspect of the present invention is a composition of ESTs representing differentially expressed genes and substantially identical nucleic acids in the presence and absence of infection in crustaceans. Any suitable crustacean can be analyzed. In one embodiment, the crustacean is a shrimp. In a preferred embodiment, the shrimp is a *P. vannamei* shrimp. Any suitable type of infection can be analyzed. In one embodiment, the infection is a viral infection. The virus can be white spot syndrome virus, Taura syndrome virus, infectious hypodermal and hematopoietic virus, yellowhead virus or baculovirus penae. In a preferred embodiment, the virus is a member of the genus *Whispovirus*. Most preferrably, the virus is white spot syndrome virus (WSSV). Differential expression of genes include, but are not limited to genes that are up or down regulated relative to the genes expressed in a non-infected crustacean.

[0059] In one embodiment, differential gene expression is determined using ESTs isolated from cDNA libraries of virally-infected shrimp and non-virally infected shrimp. The gene expression can be determined using the hepatopancreas tissue or the hemocyte population. Preferrably, the hepatopancreas tissue is the source of the ESTs. Any suitable numbers of ESTs may be considered.

[0060] Gene expression can be analyzed at any time during infection, including but not limited to time periods representing the onset of infection or the resolving of infection as well as during acute and chronic infections. In one embodiment, the EST isolated specifically correspond to the onset of infection, particularly that of viral disease. It is

contemplated that gene expression analysis may also be performed if the crustacean has more than one infective entity.

[0061] In a specific embodiment, the ESTs are isolated from shrimp that non-infected or infected with WSSV at the onset of viral infection.

[0062] Therefore, the invention provides isolated and recombinant nucleic acids, including ESTs. The invention further provides probes comprising or consisting of nucleic acids, *e.g.*, ESTs, of the invention.

[0063] The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more, residues. In one aspect, the nucleic acid encodes at least one polypeptide having a biologic activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection. In another aspect, the invention provides nucleic acids for use as probes, inhibitory molecules (*e.g.*, antisense, iRNAs), transcriptional or translational regulation, and the like. Exemplary nucleic acids of the invention include isolated or recombinant nucleic acids comprising a nucleic acid sequence as set forth in Appendix A, and subsequences thereof, *e.g.*, at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500 or more residues in length, or over the full length of a gene or transcript.

[0064] Another aspect of the invention is an isolated or recombinant nucleic acid including at least 10 consecutive bases of a nucleic acid sequence of the invention, sequences substantially identical thereto, and the sequences complementary thereto.

[0065] The invention provides isolated or recombinant nucleic acids comprising a sequence that hybridizes under stringent conditions to a nucleic acid of the invention, *e.g.*,

any one of the sequences in Appendix A. The nucleic acid can be at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500 or more residues in length or the full length of the gene or transcript. In one aspect, the stringent conditions include a wash step comprising a wash in 0.1X SSC at a temperature of about 65°C for about 15 minutes.

[0066] In a specific embodiment, the gene of interest is a lectin gene. Lectin are known to plays a critical role in the innate immunity in vertebrates by activating complement factor after binding to the carbohydrate moieties on the surfaces of viral capsid proteins (Vorup-Jensen *et al.*, 2000). In humans, the C-type lectin exhibits CD4-independent binding of the envelope glycoprotein, gp120, of human immunodefficiency virus (HIV) (Curtis *et al.*, 1992). Human MBL was also shown to bind to the envelope proteins of the influenza A virus, neutralizing the virus by inhibiting the spread of the virus and simultaneously activating the complement cascade (Kase *et al.*, 1999; Malhotra *et al.*, 1994). Replacement of MBL to MBL-deficient human has shown encouraging results in enhancing complement activation ability and opsonic activity towards *Saccharomyces cerevisae* in the treated individuals (reviewed in Kilpatric, 2002). In invertebrates (tunicate *Clavelina picta*), the binding of MBL to microbial ligands activates the complement component C3, through an MBL- associated serine proteinase. This leads to phagocytosis of the opsonized target and/ or humoral cell killing via the assembly of a membrane attack complex (Vasta *et al.*, 1999). Therefore, lectins with such activity are suitable for use in the prevention and treatment of infection in invertebrates.

[0067] The nucleic acids of the invention can be made, isolated and/or manipulated by, *e.g.*, cloning and expression of cDNA libraries, amplification of message or genomic DNA by PCR, and the like. Techniques for the manipulation of nucleic acids, such as, *e.g.*, subcloning, labeling probes (*e.g.*, random-primer labeling using Klenow polymerase, nick translation, amplification), sequencing, hybridization and the like are well described in the scientific and patent literature, see, *e.g.*, Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND

MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

[0068] The invention provides isolated or recombinant nucleic acids that hybridize under stringent conditions to an exemplary sequence of the invention, or a nucleic acid that encodes a polypeptide of the invention. The stringent conditions can be highly stringent conditions, medium stringent conditions, low stringent conditions, including the high and reduced stringency conditions described herein. In one aspect, it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention, as discussed below.

[0069] In alternative embodiments, nucleic acids of the invention as defined by their ability to hybridize under stringent conditions can be between about five residues and the full length of nucleic acid of the invention; *e.g.*, they can be at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, or more, residues in length. Nucleic acids shorter than full length are also included. These nucleic acids can be useful as, *e.g.*, hybridization probes, labeling probes, PCR oligonucleotide probes, iRNA, antisense or sequences encoding antibody binding peptides (epitopes), motifs, active sites and the like.

[0070] In one aspect, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprises conditions of about 50% formamide at about 37°C to 42°C. In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency comprising conditions in about 35% to 25% formamide at about 30°C to 35°C.

[0071] Alternatively, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprising conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and a repetitive sequence blocking nucleic acid, such as cot-1 or salmon sperm DNA (*e.g.*, 200 n/ml sheared and denatured salmon sperm DNA). In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency conditions comprising 35% formamide at a reduced temperature of 35°C.

[0072] Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate"

hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

[0073] The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Nucleic acids of the invention are also defined by their ability to hybridize under high, medium, and low stringency conditions as set forth in Ausubel and Sambrook. Variations on the above ranges and conditions are well known in the art. Hybridization conditions are discussed further, below.

[0074] The above procedure may be modified to identify nucleic acids having decreasing levels of homology to the probe sequence. For example, to obtain nucleic acids of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a Na^+ concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 55°C. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45°C.

[0075] The invention also provides nucleic acid probes that can be used, *e.g.*, for identifying nucleic acids encoding a polypeptide with a biologic activity or fragments thereof or for identifying genes. In one aspect, the probe comprises at least 10 consecutive bases of a nucleic acid of the invention. Alternatively, a probe of the invention can be at least about 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150 or about 10 to 50, about 20 to 60 about 30 to 70, consecutive bases of a sequence as set forth in a nucleic acid of the invention. The probes identify a nucleic acid by binding and/or hybridization. The probes can be used in arrays of the invention, see discussion below, including, *e.g.*, capillary arrays. The probes of the invention can also be used to isolate other nucleic acids or polypeptides.

[0076] The invention provides expression vectors and cloning vehicles comprising nucleic acids of the invention, *e.g.*, sequences encoding the recombinant proteins encoded by the nucleic acids of the invention. Expression vectors and cloning vehicles of the invention can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (*e.g.*, vaccinia, adenovirus, foul pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as bacillus, Aspergillus and yeast). In one embodiment, the expression vectors and cloning vehicle comprise yeast plasmids. Vectors of the invention can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available.

[0077] The expression vector can comprise a promoter, a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. Mammalian expression vectors can comprise an origin of replication, any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. In some aspects, DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

[0078] In one aspect, the expression vectors contain one or more selectable marker genes to permit selection of host cells containing the vector.

[0079] Vectors for expressing the polypeptide or fragment thereof in eukaryotic cells can also contain enhancers to increase expression levels. Enhancers are cis-acting elements of DNA, usually from about 10 to about 300 bp in length that act on a promoter to increase its transcription.

[0080] The invention provides cloning vehicles comprising an expression cassette (*e.g.*, a vector) of the invention or a nucleic acid of the invention. The cloning vehicle can be a viral vector, a baculovirus, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector can comprise an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle can comprise a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived

vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

[0081] The invention provides transformed cell comprising a nucleic acid of the invention or an expression cassette (*e.g.*, a vector) of the invention, or a cloning vehicle of the invention. In one aspect, the transformed cell can be a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

[0082] In another aspect, provided herein are recombinant proteins derived from full-length gene or recombinant truncated amino acids derived from the functional domain of the gene identified using the EST differential libraries provided herein. The identified ESTs of interest can be used to clone full length cDNAs using methods known in the art. *See e.g.*, U.S. Patent No. 6,265,165. Recombinant polypeptides generated from these nucleic acids can be individually isolated or cloned and tested for a desired activity. Any recombinant expression system can be used, including bacterial, mammalian, yeast, insect or plant cell expression systems. Recombinant truncated proteins of interest are those that retain at least a detectable amount of the desired biologic activity.

[0083] The invention provides fusion proteins and nucleic acids encoding them. A polypeptide of the invention can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, *e.g.*, producing a more protective peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like.

[0084] In practicing the invention, nucleic acids of the invention and nucleic acids encoding the polypeptides of the invention, or modified nucleic acids of the invention, can be reproduced by amplification. Amplification can also be used to clone or modify the nucleic acids of the invention. Thus, the invention provides amplification primer sequence pairs for amplifying nucleic acids of the invention. One of skill in the art can design amplification primer sequence pairs for any part of or the full length of these sequences.

[0085] The invention provides methods of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid of the invention operably linked to a

promoter; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide. In one aspect, the method can further comprise transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

[0086] The invention provides isolated or recombinant antibodies that specifically bind to the recombinant polypeptides of the invention. These antibodies can be used to isolate, identify or quantify the recombinant polypeptides of the invention or related polypeptides. These antibodies can be used to isolate other polypeptides within the scope the invention. The antibodies can be designed to bind to an active site of a recombinant polypeptide. Thus, the invention provides methods of inhibiting polypeptides using the antibodies of the invention.

[0087] The antibodies can be used in immunoprecipitation, staining, immunoaffinity columns, and the like. If desired, nucleic acid sequences encoding for specific antigens can be generated by immunization followed by isolation of polypeptide or nucleic acid, amplification or cloning and immobilization of polypeptide onto an array of the invention. Alternatively, the methods of the invention can be used to modify the structure of an antibody produced by a cell to be modified, *e.g.*, an antibody's affinity can be increased or decreased. Furthermore, the ability to make or modify antibodies can be a phenotype engineered into a cell by the methods of the invention.

[0088] Methods of immunization, producing and isolating antibodies (polyclonal and monoclonal) are known to those of skill in the art and described in the scientific and patent literature, see, *e.g.*, Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) Nature 256:495; Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Antibodies also can be generated in vitro, *e.g.*, using recombinant antibody binding site expressing phage display libraries, in addition to the traditional in vivo methods using animals. See, *e.g.*, Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45.

[0089] Polyclonal antibodies generated against the polypeptides of the invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to a non-human animal. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

[0090] For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique, the trioma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (see, *e.g.,* Cole (1985) in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Uses of Recombinant Nucleic Acids and Polypeptides

Methods of treating infection

[0091] Another aspect of the invention is a method of protecting crustacean from infection using recombinant protein or nucleic acids derived from the full-length gene or recombinant truncated protein or nucleic acids derived from the functional domain of the gene identified using the EST differential libraries of the instant invention. In one embodiment, the infection is a viral infection. In a specific embodiment, the viral infection is WSSV. In one embodiment, the crustacean is a shrimp. The recombinant protein or nucleic acid, or biologically active fragment thereof, can be administered in any suitable manner in a single dose or repeatedly. The recombinant protein or nucleic acid can be administered alone or in combination with other treatment modalities for the infection. A therapeutically effective amount of recombinant polypeptide or biolgically active fragment thereof is an amount that reduces or eliminates at least one symptom of the infection. In one embodiment, a therapeutically effective amount prevents infection or cures established infection.

[0092] In one aspect of the present invention, the crustacean can be provided a feed or feed supplement which incorporates recombinant protein or nucleic acids identified using the EST differential libraries of the instant invention as a prophylactic or therapeutic treatment for the deleterious effects of the virus on the host. Thus, the present invention

provides for a composition comprising at least one recombinant protein or nucleic acid, or biologically active fragment thereof, in a food source for the crustacean. The invention provides edible enzyme delivery matrices comprising a polypeptide of the invention, *e.g.*, a polypeptide encoded by the nucleic acid of the invention.

[0093] The bioactive food complex can be preserved without drying as a semi-solid, moist paste, not a liquid, composed of microcapsules or beads, or as moist noodles, pellets, sheets or other forms, or can be stored frozen by employing cryopreservatives. The bioactive food complex can be added directly to aquaculture animal containment systems to be eaten by aquatic animals. In one embodiment of the invention, the bioactive food complex or the primary emulsion of the bioactive food complex can be added to pelleted or extruded aquatic feeds as a top-dress coating or enrobing of the pelleted or extruded aquatic feed.

[0094] The foodstuffs incorporated into the particulate fish foods of this invention may be those which are normally used for other particulate fish foods. Frequently fish meal will provide at least 30% of the formulation and often it will provide at least 50% of the formulation.

[0095] Particulate fish foods are customarily manufactured by three routes, all of which involve some form of extrusion through a die. The methods are normally classified as compressed pelleting (also known as steam pelleting),/extruded pelleting and moist pelleting.

[0100] In one embodiment, amplified cDNA can be cloned into bacterial expression vector (such as pET100 Directional TOPO Expression vector, Invitrogen Inc.) and used to transform BL 21 Star (DE3) One Shot Chemically Competent *Escherichia coli* (Invitrogen, Inc.) following the manufacturer's protocol. *Escherichia coli* strain BL21 cells carrying IPTG inducible gene can be grown in LB medium containing ampicillin, and subsequently induced with IPTG to elicit expression of recombinant protein. The expression of the recombinant protein will be empirically optimized to obtain maximum induction using routine methods. Bacterial biomass containing cells, which express the shrimp recombinant protein, are then added to shrimp feed in a free or microbound format (in beads composed of alginate and starch in a polymeric form). Attractants can optionally be added to make the

feed or beads more palatable to the target species. In the case of shrimp, krill meal is employed as the attractant.

RNAi reagents

[0101] In one aspect, the invention provides an RNA inhibitory molecule, a so-called "RNAi" molecule, comprising a recombinant protein or nucleic acid sequence of the invention to be administered as a prophylactic or therapeutic regimen for infection. The RNAi molecule comprises a double-stranded RNA (dsRNA) molecule. The RNAi can inhibit expression of a gene encoding a nucleic acid of the invention. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. While the invention is not limited by any particular mechanism of action, the RNAi can enter a cell and cause the degradation of a single-stranded RNA (ssRNA) of similar or identical sequences, including endogenous mRNAs. When a cell is exposed to double-stranded RNA (dsRNA), mRNA from the homologous gene is selectively degraded by a process called RNA interference (RNAi). A possible basic mechanism behind RNAi is the breaking of a double-stranded RNA (dsRNA) matching a specific gene sequence into short pieces called short interfering RNA, which trigger the degradation of mRNA that matches its sequence. In one aspect, the RNAi's of the invention are used in gene-silencing therapeutics, see, *e.g.,* Shuey (2002) Drug Discov. Today 7:1040-1046. In one aspect, the invention provides methods to selectively degrade RNA using the RNAi's of the invention. The process may be practiced *in vitro*, *ex vivo* or *in vivo*. In one aspect, the RNAi molecules of the invention can be used to generate a loss-of-function mutation in a cell, an organ or an animal. Methods for making and using RNAi molecules for selectively degrade RNA are well known in the art, see, *e.g.,* U.S. Patent No. 6,506,559; 6,511,824; 6,515,109; 6,489,127.

Functional genomics studies

[0102] In yet another aspect of the present invention, a library of ESTs as well as cDNAs are provided for use in functional genomic analysis. In practicing the methods of the invention, a variety of apparatus and methodologies can be used to in conjunction with the polypeptides and nucleic acids of the invention, *e.g.,* to screen polypeptides for biologic activity, to screen compounds as potential modulators, *e.g.,* activators or inhibitors, of an activity of a recombinant polypeptide encoded by a nucleic acid of the invention, for

antibodies that bind to a polypeptide of the invention, for nucleic acids that hybridize to a nucleic acid of the invention, to screen for cells expressing a polypeptide of the invention and the like.

[0103] Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (*e.g.*, small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. For example, in one aspect of the invention, a monitored parameter is transcript expression of a gene. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the invention. Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins. The present invention can be practiced with any known "array," also referred to as a "microarray" or "nucleic acid array" or "polypeptide array" or "antibody array" or "biochip," or variation thereof. Arrays are generically a plurality of "spots" or "target elements," each target element comprising a defined amount of one or more biological molecules, *e.g.*, oligonucleotides, immobilized onto a defined area of a substrate surface for specific binding to a sample molecule, *e.g.*, mRNA transcripts.

[0104] In practicing the methods of the invention, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as described, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, *e.g.*, WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, *e.g.*, Johnston (1998) Curr. Biol. 8:R171-R174; Schummer (1997) Biotechniques 23:1087-1092; Kern (1997) Biotechniques 23:120-124; Solinas-Toldo (1997) Genes, Chromosomes & Cancer 20:399-407; Bowtell (1999) Nature

Genetics Supp. 21:25-32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Biopesticides

[0105] In another aspect of the invention the differentially expressed ESTs differentially with viral infection are useful as a biopesticide. For example, baculovirus expressing shrimp chitinase gene can be used as a biological agent to infect and kill lepidopteran insects that infect agricultural crops. Baculoviruses have been widely used as a biological control agent in agricultural crop pest management. Further effectiveness can be achieved by co-expressing shrimp chitinase gene with the recombinant polypeptide or biologically active fragment thereof, and then spraying this recombinant baculovirus on crop fields infected by an insect pest.

Screening assays

[0106] The invention provides methods for identifying a substrate of a recombinant polypeptide of interest, comprising the following steps: (a) providing a polypeptide of the invention; or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test substrate; and (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as the polypeptide substrate.

[0107] The invention also provides methods of determining whether a test compound specifically binds to a polypeptide comprising the following steps: (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid comprises a nucleic acid of the invention, or, providing a polypeptide of the invention; (b) providing a test compound; (c) contacting the polypeptide with the test compound; and (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

[0108] The invention provides methods for identifying a modulator of a recombinant polypeptide's activity comprising the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test compound; (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the recombinant polypeptide, wherein a change in the recombinant

polypeptide activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the recombinant polypeptide activity. In one aspect, the recombinant polypeptide activity can be measured by providing an recombinant polypeptide substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product. A decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of recombinant polypeptide activity. An increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of recombinant polypeptide activity.

Diagnostic Kits

[0109] In another aspect of the present invention, methods are provided that use the differentially expressed genes identified in the EST library as a diagnostic tool to evaluate crustaceans in aquaculture and in food processing. Also provided herein is a kit comprising at least one differentially expressed gene as a nucleic acid or recombinant polypeptide, wherein the kit can be used to positively identify a crustacean as being infected or non-infected with the pathogen of interest. Such kits are also useful for monitoring the course of infection. In one embodiment, the kit comprises at least one antibody or antigen binding fragment thereof that binds the polypeptide encoded by the EST of interest. The kit can be in any suitable format, and in some embodiments includes instructions for use.

Examples

[0110] The invention, as contemplated herein, is described in the following examples for exemplification purposes only and is not intended to limit the scope of the invention.

Example 1. Isolation of expressed sequence tags (ESTs).

[0111] ESTs have been isolated from cDNA libraries of healthy and WSSV-infected shrimp hepatopancreas tissue. Two cDNA libraries were constructed from hepatopancreas

tissues of healthy (PvH) and WSSV-infected (PvW) *P. vannamei* shrimp using SMART cDNA amplification method (BD Bioscience, California) and cloned into a plasmid vector (pAL16, Evrogen, Inc., Moscow, Russia). Randomly selected recombinant clones were sequenced using vector-derived primers (SP6 and T7). A total of 1248 clones from PvH and 1152 clones from PvW library were sequenced.

[0112] Complementary DNA sequences from both libraries were edited and assembled using the programs Phred, CodonCode Aligner and Phrap. Vector sequences were removed from the sequence of each clone and ESTs having Phred score of >20 were taken for BLAST search. A total of 1248 clones from PvH and 1152 clones from PvW library were sequenced. Seventy five percent of the PvH clones (940 out of 1248 PvH clones; Phred score>20, average length 473 bases) and seventy three percent of the PvH clones (840 out of 1152 PvW clones; Phred score>20, average length 440 bases) were taken for further analysis. PvH clones represented 269 singletons and 124 contigs (each contig contains between 2-20 clones), whereas the PvW clones represented 281 singletons and 211 contigs (each contig contains 2-20 clones). Among the PvH clones, 38.7% of the singletons (105/269) and 71.4% of the contigs (90/124) showed significant similarity to known genes from other organisms by BLAST search ($e>10^{-3}$). For the PvW clones, 44.0% of the singletons (123/281) and 54.5% of the contigs (115/211) showed similarity to GenBank entries by BLAST search ($e>10^{-3}$). The remainder of the clones from both libraries showed no similarity to the database entries, and thus may represent novel genes. Combining the ESTs from healthy and WSSV-infected shrimp cDNA libraries, there were a total of 683 unigenes (Figure 3).

[0113] The average GC content of the PvH clones was 0.427±0.005 and for the PvW clones was 0.424±0.005. Based on gene homology, the PvH and PvW unigene sets were categorized into the following nine functional classes following published protocol (Adams *et al.*, 1995): Cell division, cell signaling/ communication, cell structure, cell defense, gene/protein expression, metabolism, ribosomal proteins, unknown genes and no matches (Fig. 1, Table 1). PvW library contained a significantly higher number of cell defense genes compared to the PvH library (19.4% vs. 8.1%), whereas the PvH library contained a significantly higher number of metabolic genes (37.5% vs. 25.6%) (Fig.1). A total of 40 immune genes were identified from both libraries (Table 2). A list of these immune genes,

along with their frequencies in the healthy and WSSV-infected libraries and similarities with the GenBank database entries, is provided in Table 2. The expression profile indicates a differential expression of these genes in healthy and WSSV-infected shrimp.

Table 1. Functional classes of genes isolated from hepatopancreas cDNA libraries of healthy and WSSV infected shrimp (*P. vannamei*) that showed homology with existing GenBank database entries.

Function Classes	Percent ESTs	
	Healthy Library	WSSV Infected Library
Cell Division	3.75	5.55
Cell Signaling/Communication	10.00	7.77
Cell Structure	18.75	11.11
Cell Defense	8.13	19.44
Gene/Protein Expression	7.50	7.22
Metabolism	37.50	25.55
Ribosomal Proteins	11.88	15.55
Others	2.50	7.77

Table 2. Summary of immune genes isolated from healthy and WSSV-infected shrimp hepatopancreas cDNA libraries.

Clone ID#	Gene	e-value	Frequency	
			Healthy	Infected
PvH1A02	Gamma-interferon inducible lysosomal thiol reductase precursor	6.00E-13	0	1
PvHB11	Anti-lipopolysaccharide factor	4.00E-10	0	1
PvHC06	O-sialoglycoprotein endopeptidase	3.00E-14	0	1
PvH01A03	Peritrophin 1	6.00E-04	3	2
PvH02H11	Placental protein 11 precursor	3.00E-09	1	0
PvH04A12	Mast cell carboxypeptidase A precursor	8.00E-06	1	0
PvH04G08	Low-density lipoprotein receptor-related protein precursor	9.00E-17	2	0
PvH05A11	Lysozyme	1.00E-15	12	8
PvH05H07	Sarcoplasmic calcium-binding protein, alpha-B and -A chains	2.00E-14	1	1
PvH06B03	Metaxin 2	8.00E-45	1	0
PvH06C07	Mucin-like protein 1	6.00E-07	1	0
PvH09A06	C-type lectin receptor	7.00E-04	1	0
PvH09H12	Platelet-endothelial tetraspan antigen 3	4.00E-16	1	2

Clone ID#	Gene	e-value	Frequency	
			Healthy	Infected
PvH10B06	Metallothionein	5.00E-04	1	0
PvH11G05	Keratinocytes associated protein 2	1.00E-26	1	0
PvH12B07	Thermosome beta subunit	8.00E-58	1	0
PvH13C04	Lectin 1 (Fragment)	5.00E-09	1	0
PvW03G04	Peritrophin-like protein 1	7.00E-78	1	5
PvW04C06	Ubiquitin	8.00E-37	0	2
PvW04D01	Mannose-P-dolichol utilization defect 1 protein	2.00E-26	0	1
PvW04E03	P-selectin precursor	5.00E-03	0	1
PvW04E08	Glutamate carboxypeptidase-like protein	1.00E-67	0	2
PvW04F07	Prophenoloxidase-activating proteinase-2	2.00E-04	0	1
PvW04G01	Innexin Inx3	1.00E-04	0	1
PvW05C11	Peritrophin-like protein 2	3.00E-60	0	2
PvW05D04	Peritrophin-95 precursor	8.00E-05	0	1
PvW05G02	Hepatocellular carcinoma-associated antigen 59	1.00E-33	0	1
PvW05G04	T-cell activation protein	3.00E-68	0	1
PvW07A09	SelT-like protein precursor	8.00E-17	0	1
PvW07H04	Glutathione peroxidase	1.00E-56	0	1
PvW08B06	Interleukin enhancer-binding factor 3	2.00E-05	0	1
PvW08E09	NF-kappaB essential modulator	5.00E-04	0	1
PvW08G01	Apolipoprotein D precursor	1.00E-10	3	1
PvW08G07	Thrombospondin 3 precursor	5.00E-39	0	2
PvW09E11	Chitinase	3.00E-68	3	1
PvW09G07	Nose resistant to fluoxetine protein 6 precursor	5.00E-11	0	1
PvW09H06	Chitinase precursor	3.00E-06	1	1
PvW10A02	Peroxiredoxin	1.00E-60	0	2
PvW10D06	Heat shock protein STI1	8.00E-14	0	1
PvW11A05	Tetraspanin 2	3.00E-22	0	1

Table 2. (Cont) Right hand columns of the above section of Table 2. Correspond line for line to the other section.

Frame	nt	Score	Similarity	Species	Accession #
+ 3	312-740	76.6	50%	*Amblyomma americanum*	AAK82985
+1	412-597	66.6	67%	*Atlantic horseshoe crab*	A23931
+3	96-545	80.5	53%	*Methanococcus jannaschii*	A54441
+2	203-406	46.6	50%	*Mamestra configurata*	AAP33177

Frame	nt	Score	Similarity	Species	Accession #
-3	468-217	63.2	55%	*Mus musculus*	NP_032928
+1	130-336	53.1	58%	*Homo sapiens*	AAH12613
+1	91-546	89.4	40%	*Rattus norvegicus*	NP_786938
+1	238-567	85.1	51%	*Bathymodiolus azoricus*	AAN16208
-1	734-507	81.3	60%	*Penaeus sp.*	SCPA_PENSP
+1	184-663	182	70%	*Mus musculus*	MTX2_MOUSE
+1	118-423	56.6	42%	*Ctenocephalides felis*	AAM21357
-2	674-324	46.2	41%	*Paralabidochromis chilotes*	AAP58738
+2	200-667	83.2	45%	*Homo sapiens*	C151_HUMAN
-1	419-288	47	50%	*Homarus americanus*	MT1_HOMAM
+2	236-583	122	67%	*Homo sapiens*	NP_776251
+2	278-868	225	75%	*Homo sapiens*	AAB67249
+1	142-564	63.5	44%	*Girardia tigrina*	AAL29940
+2	131-703	292	78%	*Penaeus semisulcatus*	AAF34331
+2	116-466	155	70%	*Branchiostoma belcheri tsingtaunese*	AAL55470
+3	180-653	117	56%	*Mus musculus*	MPU1_MOUSE
+3	390-614	43.9	54%	*Rattus norvegicus*	NP_446205
+2	197-829	258	72%	*Homo sapiens*	CAC69883
+2	116-440	48.1	66%	*Manduca sexta*	AAL76085
+3	267-680	48.9	40%	*Drosophila melanogaster*	NP_524730
+2	164-694	233	69%	*Penaeus semisulcatus*	AAF34332
-2	546-211	49.7	40%	*Lucilia cuprina*	AAB70878
-1	722-260	145	63%	*Homo sapiens*	AAH17570
+2	95-514	259	85%	*Litopenaeus vannamei*	AAN74647
+2	479-658	89	76%	*Homo sapiens*	NP_057359
-2	742-197	221	74%	*Homo sapiens*	CAA48394
+2	266-511	51.6	59%	*Homo sapiens*	NP_004507
+3	102-560	46.6	44%	*Mus musculus*	AAC40153
-2	575-369	68.9	65%	*Homo sapiens*	NP_001638
+1	223-888	163	49%	*Penaeus monodon*	AAN17670
+2	95-514	259	85%	*Litopenaeus vannamei*	AAN74647
-1	766-401	70.1	49%	*Caenorhabditis elegans*	NP_507120
+1	265-483	53.5	44%	*Tenebrio molitor*	CAD31740
+1	214-696	235	82%	*Haemaphysalis longicornis*	BAB17604
-3	766-578	79	76%	*Drosophila melanogaster*	NP_477354
+1	358-627	87.8	65%	*Drosophila melanogaster*	NP_523515

Example 2. Confirmation of differential expression of shrimp ESTs.

[0114] As a proof of principle, the differential expressions of three shrimp ESTs were confirmed by examining their mRNA expression in healthy and WSSV-infected shrimp by real-time RT-PCR assay. These genes include a λ interferon inducible lysosomal thiol reductase (GILT) gene, a lectin gene named P-selectin, and a chitinase gene.

[0115] The predicted amino acid sequences of the shrimp λ interferon inducible lysosomal thiol reductase (GILT) gene, a lectin gene named P-selectin, and a chitinase gene and their similarity with the GenBank database entries are given below (Tables 3-5).

Table 3. The similarity between shrimp λ interferon inducible lysosomal thiol reductase (GILT) gene and homologous genes in the GenBank database.

Amino acid sequence of shrimp λ interferon inducible lysosomal thiol reductase (GILT) gene:

MSGPHVGAGXRLTSGINAEYAGTQLVLAMKAHTTLTALATVLAWLSLAESAKPVTVSVYYESLCPDSQRFVVTQ
LYPVWQDLKEIMLLDVNSYGKSKDTPAGDGYTFECQNGPDECEGNIMLTCAKKYSSEEQYMSFANCTMAELVGT
AAGARCAEVSGVNYTRVYDCFNSVEGQQLQHEVGVKQAQFXPPLNFVPWILINEVFTERTVRRQLRTTSGKLCC
KLMRCQARKCSYRRISVYNGLV

Homology comparison:

	Similarity	Identity	e-value
Mouse	46%	31%	6e-15
Rat	48%	32%	3e-15
Human	48%	27%	4e-12
Branchiostoma	51%	32%	7e-22
Tick	50%	28%	6e-13
Fruitfly	48%	33%	2e-23
Mosquito	47%	33%	8e-17
Worm	50%	29%	2e-15
Caenorhabditis	50%	31%	3e-16

Table 4. The similarity between the shrimp chitinase gene and homologous genes in the GenBank database.

Amino acid sequence of shrimp chitinase gene:

MLPPHGGRGNRFSSGSTQSTRGGRSSAEYAKSKGLAGTMVWSVETDDFRGLCHNRKYHLIKTMVEVFGGGSITE PPPLPTTTRDPNEPTTTTRAPPPPGVHCTQPGLNPDPLDCTHYYLCSLNTSGGYNEKEEVCPEGTLYNPQSYYC DWASSVCHLGEDVCPNDC*

Homology comparison:

	Similarity	Identity	e-value
Tsetse Fly	43%	30%	1e-08
Fruitfly	37%	29%	9e-08
Polydnavirus	42%	29%	2e-13
Spider	48%	33%	7e-05
Mosquito	36%	27%	4e-11
Tick	35%	25%	1e-06
Mouse	40%	28%	6e-06

Table 5. The similarity between shrimp P-selectin gene and the homologous genes in the GenBank database.

Amino acid sequence of shrimp P-selectin gene:

MVDLQAAANSLVIKQWYQRRVLFWGWCFFFFYVVFIKNRTREPFAFPAKTLRSILPTDGTVGRGVAGDVDEIVT DGFLHDGAVLVGGFRGRVDPPEGGAKPDYLVGLHESPVSFVSVVRDAHPVIVLVQTVCVDVLYDIPEEVAVQAG LEPDDVSAVGLAEGADLVESPRLEGVDVDAVASDGRVGVGALLASSGRDCKHNEQGSDVKKPVHGGEHTKVAPR TLR

Homology comparison:

	Similarity	Identity	e-value
Monkey	44%	22%	1e-02
Macaque	44%	22%	1e-02
Chimpaneze	44%	21%	3e-02
Rat	54%	31%	5e-03

[0116] In order to measure the mRNA expression of these three genes in healthy and WSSV-infected shrimp, primers were designed using Primer Express software (Applied Biosystem, Inc.). The list of primers is given below (Table 6).

Table 6. List of primers used for measuring the expression of immune genes in healthy and white spot syndrome virus (WSSV) infected shrimp (*Penaeus vannamei*) by real-time RT-PCR.

Shrimp Gene	Primer Name	Primer Sequence (5'-3')
Gamma-interferon inducible lysosomal thiol reductase (GILT)	PvH1A02	FOR: TGGCAAGACCTCAAGGAAATCA REV: CGCATTCGTCTGGTCCATTTT
P-selectin	PvW4E03	FOR: CCATCGGAGGCTACTGCATCTA REV: GCCTCGAACCAGATGATGTCTC
Chitinase	PvW9E11	FOR: ACTACCTGTGCTCGCTCAACAC REV: AAGCCCAATCGCAGTAGTAGCT

[0117] The real-time RT-PCR assay showed that shrimp λ interferon inducible lysosomal thiol reductase (GILT) , P-selectin, and the chitinase genes showed differential expression between healthy and WSSV-infected shrimp. The mRNA expression of λ interferon inducible lysosomal thiol reductase (GILT) gene was down-regulated, where as the expression of chitinase and P-selectin was up-regulated in WSSV-infected shrimp. The real-time PCR assay was performed using 5 healthy and 5 WSSV-infected laboratory challenged shrimp. The summary of the real-time RT-PCR assay for each of the three genes is provided below (Tables 7-9).

Table 7. Relative quantification of λ interferon inducible lysosomal thiol reductase (GILT) mRNA expression in healthy and white spot syndrome virus-infected shrimp (*Penaeus vannamei*) by real-time RT-PCR.

Sample #	Average ΔCT ± SD	Sample #	Average ΔCT ± SD	Average ΔΔCT	Fold Changes ($2^{\Delta\Delta CT}$)
H1	5.533 ± 0.267	W1	6.333 ± 2.067	-2.507	-5.7
H2	6.100 ± 0.300	W2	10.133 ± 1.733		
H3	5.600 ± 0.200	W3	8.067 ± 0.333		
H4	5.767 ± 0.033	W4	9.933 ± 1.533		
H5	6.233 ± 0.433	W5	7.300 ± 1.100		

H1 to H5 represents healthy animals, and W1 to W5 represents WSSV-infected animals. CT represents threshold PCR cycle number. ΔCT represents the normalized Ct value. Normalized Ct value is obtained by subtracting the CT value of RT-PCR internal control gene, shrimp elongation factor-1alpha, from the CT value of the target gene. ΔΔCT obtained by subtracting the ΔCT of healthy from the ΔCT value for the WSSV-infected sample. Fold changes indicates the changes in target gene expression in the WSSV-infected compared to the healthy animals.

Table 8. Relative quantification of chitinase mRNA expression in healthy and white spot syndrome virus-infected shrimp (*Penaeus vannamei*) by real-time RT-PCR.

Sample #	Average ΔCT ± SD	Sample #	Average ΔCT ± SD	Average ΔΔCT	Fold Changes ($2^{\Delta\Delta CT}$)
H1	1.100 ± 0.040	W1	-0.300 ± 0.740	0.700	1.62
H2	1.167 ± 0.027	W2	0.967 ± 0.967		
H3	1.200 ± 0.060	W3	0.033 ± 0.407		
H4	0.367 ± 0.773	W4	1.267 ± 1.227		
H5	1.900 ± 0.760	W5	0.233 ± 0.260		

H1 to H5 represents healthy animals, and W1 to W5 represents WSSV-infected animals. CT represents threshold PCR cycle number. ΔCT represents the normalized Ct value. Normalized Ct value is obtained by subtracting the CT value of RT-PCR internal control gene, shrimp elongation factor-1alpha, from the CT value of the target gene. ΔΔCT obtained by subtracting the ΔCT of healthy from the ΔCT value for the WSSV-infected sample. Fold changes indicates the changes in target gene expression in the WSSV-infected compared to the healthy animals.

Table 9. Relative quantification of P-selectin mRNA expression in healthy and white spot syndrome virus-infected shrimp (*Penaeus vannamei*) by real-time RT-PCR.

Sample #	Average ΔCT ± SD	Sample #	Average ΔCT ± SD	ΔΔCT	Fold Changes ($2^{\Delta\Delta CT}$)
H1	3.967 ± 0.733	W1	3.567 ± 0.033	1.100	2.11
H2	4.667 ± 0.033	W2	4.700 ± 1.100		
H3	5.167 ± 0.467	W3	2.100 ± 1.500		
H4	4.267 ± 0.433	W4	4.133 ± 0.533		
H5	5.433 ± 0.733	W5	3.533 ± 0.067		

H1 to H5 represents healthy animals, and W1 to W5 represents WSSV-infected animals. CT represents threshold PCR cycle number. ΔCT represents the normalized Ct value. Normalized Ct value is obtained by subtracting the CT value of RT-PCR internal control gene, shrimp elongation factor-1alpha, from the CT value of the target gene. ΔΔCT obtained by subtracting the ΔCT of healthy from the ΔCT value for the WSSV-infected sample. Fold

changes indicates the changes in target gene expression in the WSSV-infected compared to the healthy animals.

Example 3. Cloning of shrimp ESTs.

[0118] In order to amplify the full-length open reading frame (ORF) in shrimp ESTs, primers will be designed using primer express software (Applied Biosystems, Inc.). Primers will also be designed to amplify a truncated version of the gene (representing the functional domain only). Amplified cDNA will be cloned into bacterial expression vector (such as pET100 Directional TOPO Expression vector, Invitrogen Inc.) and used to transform BL 21 Star (DE3) One Shot Chemically Competent *Escherichia coli* (Invitrogen, Inc.) following manufacturer's protocol.

Example 4. Bacterial expression of shrimp recombinant protein and formulation into feed.

[0119] The expression of the recombinant protein will be either constitutive or under an induction system (*e.g.,* induced by IPTG) and empirically optimized to obtain maximum protein expression. *Escherichia coli* strain BL21 cells carrying IPTG inducible gene will be grown in LB medium containing ampicillin, and then will be induced with IPTG for the expression of recombinant protein. Bacterial biomass containing cells, which express the shrimp recombinant protein, will be added to shrimp feed in a free or microbound format (*e.g.,* in beads composed of alginate and starch in a polymeric form). Attractants are added to make the feed or beads more palatable to the target species (in the case of shrimp, krill meal would be a good attractant).

Example 5. Method for protection of shrimp from WSSV infection.

[0120] Shrimp are fed either a control diet or a diet containing bacterial biomass expressing recombinant protein (Example 4). Animals are challenged with WSSV, and their survivability in response to viral infection will be measured. The WSSV load in the control and treatment samples is measured by real-time PCR following published protocol (Dhar *et al.* 2001). The mRNA expression of the target gene will be measured in the treated and control animals using real-time RT-PCR to determine the difference in expression in two treatment groups following the published method (Dhar *et al.* 2003). Protection from viral

challenge will be determined by an increased survival versus a control not fed the diet containing the WSSV recombinant protein.

Example 6. DNA method for protection of shrimp from WSSV infection.

[0121] Shrimp are immersed in a medium containing nucleic acid encapsulated in a liposome. The DNA will be designed to express proteins that are upregulated during infection by WSSV as determined from the differential EST libraries. The DNA is then absorbed through the gills or through gut lining and will then expressing the proteins in the tissues that absorb the DNA and thus providing protection against WSSV.

Example 7. RNAi method for protection of shrimp from WSSV infection.

[0122] A portion of a gene or genes identified using a differential expression library from shrimp (healthy versus WSSV-infected) as described in the specification are used to model a small interfering RNA as described by Timmons et al (2001). This DNA is then fed to the shrimp via incorporation in a feed or feed supplement. As shown for *C. elegans* in previous research (Timmons *et al.* 2001), this can interfere with expression of specific genes. Genes that are up-regulated in the EST differential library that have no defense function maybe important to viral infection and will be down regulated using this RNAi and this will suppress or inhibit WSSV infection.

Example 8. Diagnostic for WSSV infection.

[0123] The differentially expressed genes that are tied directly to WSSV infection, or general viral infection, will be used in a multiplexed assay to determine the metabolic health of a cultured shrimp population. Compared to WSSV titer determination, which tell the relative amount of virus in the population, this diagnostic will determine the effect of the infection on the metabolic health of the population (progress of the disease compared to the growth of the pathogen). Metabolic genes will be chosen from the EST library that are most influenced in the early, mid, and late stage infection. Defense genes will be chosen from the EST libraries that are the best indicators of early, mid, and late stage infection. These genes will be synthesized with a fluorescent tag on one end and a quencher on the other end as previously described for the molecular beacon system (Cantor 1996; Tyagi and Kramer

1996; Little and Vonk 2000; Livak *et al.* 2000; Tyagi *et al.* 2000). These will be printed on a chip and then a crude DNA preparation from the infected shrimp used for hybridization of the chip. The detection will be carried out on a chip reader and monitored for the appearance of fluorescence and quantity of fluorescence relative to total DNA. This will be correlated empirically to disease progression and used as a way to monitor the disease status of the animals. This will be particularly important as disease resistant lines of shrimp are developed.

Example 9. Method for protection of shrimp from Taura syndrome virus (TSV) infection.

[0124] ESTs isolated from the healthy and WSSV-infected shrimp can be used for developing therapeutics for not only WSSV but also for other viral diseases such as Taura syndrome disease caused by the Taura syndrome virus (TSV). Taura disease, caused by the TSV, has caused catastrophic losses in the Western Hemisphere, and more recently in Taiwan (Dhar *et al.*, 2004). The cDNA libraries described in Example 1 contained immune genes such as the low-density lipoprotein receptor (LDLr) gene (Table 2) that could be for developing therapeutics against the TSV. In addition to binding and internalizing lipoprotein molecules from circulating haemolymph, LDLr is known to be involved in viral pathogenesis. For example, Hepatitis C virus has been shown to complex with LDL and interacts with the LDL receptor (Wunschmann *et al.*, 2000). Type 2 rhinovirus was shown to up-regulate LDL receptor expression on human tracheal epithelial cells (Suzuki *et al.*, 2001) and binding of the HIV-transactivator protein is mediated by LDL-related protein (Liu *et al.*, 2000).

[0125] Primers will be designed to amplify the full-length open reading frame (ORF) of shrimp LDLr using primer express software (Applied Biosystems, Inc.). Primers will also be designed to amplify a truncated version of the gene (representing the functional domain only). Amplified cDNA will be cloned into bacterial expression vector (such as pET100 Directional TOPO Expression vector, Invitrogen Inc.) and used to transform BL 21 Star (DE3) One Shot Chemically Competent *Escherichia coli* (Invitrogen, Inc.) following manufacturer's protocol. *Escherichia coli* strain BL21 cells carrying IPTG inducible gene will be grown in LB medium containing ampicillin, and then will be induced with IPTG for the expression of recombinant protein. The expression of the recombinant protein will be

empirically optimized to obtain maximum induction. Bacterial biomass containing cells, which express the shrimp recombinant protein, will be added to shrimp feed in a free or microbound format (in beads composed of alginate and starch in a polymeric form). Attractants are added to make the feed or beads more palatable to the target species (in the case of shrimp, krill meal would be a good attractant).

[0126] Animals will be challenged with TSV, and their survivability in response to viral infection will be measured. The TSV load in the control and treatment samples is measured by real-time PCR following published protocol (Dhar *et al.* 2002). The mRNA expression of the target gene will be measured in the treated and control animals using real-time RT-PCR to determine the difference in expression in two treatment groups following published method (Dhar *et al.* 2003). Protection from viral challenge will be determined by an increased survival versus a control not fed the diet containing the TSV recombinant protein.

Example 10. Use of recombinant lectins for controlling viral and bacterial diseases of shrimp and other aquaculture species.

[0127] The differentially expressed genes in shrimp included a number of lectin genes (see Table 2, Gene ID# PvH09A06, PvH13C04, and PvW04E03. Primers will be designed to amplify the full-length open reading frame (ORF) of shrimp lectins using primer express software (Applied Biosystems, Inc.). Primers will also be designed to amplify a truncated version of the gene (representing the functional domain only). Amplified cDNA will be cloned into bacterial expression vector (such as pET100 Directional TOPO Expression vector, Invitrogen Inc.) and used to transform BL 21 Star (DE3) One Shot Chemically Competent *Escherichia coli* (Invitrogen, Inc.) following manufacturer's protocol. *Escherichia coli* strain BL21 cells carrying IPTG inducible gene will be grown in LB medium containing ampicillin, and then will be induced with IPTG for the expression of recombinant protein. The expression of the recombinant protein will be empirically optimized to obtain maximum induction. Bacterial biomass containing cells, which express the shrimp recombinant protein, will be added to shrimp feed in a free or microbound format (in beads composed of alginate and starch in a polymeric form). Attractants are added to make the feed or beads more palatable to the target species (in the case of shrimp, krill meal would be a good attractant).

[0128] Animals will be challenged with WSSV, TSV, yellohead virus (YHV), and infectious hypodermal and hematopoietic virus (IHHNV) and bacterial pathogens such as *Vibrio sp. including V. penaeicida*, an important bacterial pathogen of shrimp. The survivability of shrimp after viral and bacterial challenge will be recorded. The viral load (WSSV, TSV, YHV and IHHNV) will be measured by real-time PCR following published protocol (Dhar *et al.*, 2001, 2002). The mRNA expression of the lectin gene in the virus and bacterial-challenged animals will be measured in the treated and control animals using real-time RT-PCR to determine the difference in expression in two treatment groups following published method (Dhar *et al.* 2003). Protection from viral challenge will be determined by an increased survival versus a control not fed the diet containing recombinant lectin.

[0129] In addition, by using homology modeling the tertiary structure of shrimp lectin genes will be determined. Shrimp lectins mentioned in see Table 2, Gene ID# PvH09A06, PvH13C04, and PvW04E03 will be taken for determining the tertiary structure by comparing with the crystal structure of homologous lectin available in the GenBank database (Feinberg *et al.*, 2001). Homology modeling will enable to determine the sugar binding pocket. Ligand binding assay,by virtual docking of different ligands (sugars), will be performed, and the affinity of different lectins to different sugars will be determined. Based on sugar binding affinity, recombinant lectins could be used as feed supplement to protect shrimp and other aquaculture species from different viral and bacterial disease.

Example 11. Method for protection of shrimp and other aquaculture species from bacterial diseases using recombinant anti-lipopolysaccharide protein.

[0130] Analysis of shrimp EST sequence revealed the presence of an anti-lipopolysaccharide (Anti-LPS) gene (see Table 2 EST ID #PvHB11). Anti-LPS gene encodes for small a basic protein that binds and neutralizes LPS, and thus possesses a strong antibacterial effect on the growth of Gram-negative bacteria (Iwanaga et al., 1992; Ried et al., 1996). Primers will be designed to amplify the full-length open reading frame (ORF) of shrimp anti-LPS gene using primer express software (Applied Biosystems, Inc.). Primers will also be designed to amplify a truncated version of the gene (representing the functional domain only). Amplified cDNA will be cloned into bacterial expression vector (such as pET100 Directional TOPO Expression vector, Invitrogen Inc.) and used to transform BL 21

Star (DE3) One Shot Chemically Competent *Escherichia coli* (Invitrogen, Inc.) following manufacturer's protocol. *Escherichia coli* strain BL21 cells carrying IPTG inducible gene will be grown in LB medium containing ampicillin, and then will be induced with IPTG for the expression of recombinant protein. The expression of the recombinant protein will be empirically optimized to obtain maximum induction. Anti-LPS recombinant protein will be purified from the recombinant cells and its Gram-negative bacterial neutralizing activity will be assayed against a number of Gram-negative bacterial pathogens of shrimp, fish and terrestrial agricultural species e. g. *Vibrio penaeicida, Vibrio anguillarium, Virio parahemolyticus, Lactococcus garviae, Pasteurella piscicida.*

[0131] Bacterial biomass containing cells, which express the shrimp recombinant anti-LPS protein, will be added to shrimp feed in a free or microbound format (in beads composed of alginate and starch in a polymeric form). Attractants are added to make the feed or beads more palatable to the target species (in the case of shrimp, krill meal would be a good attractant). Shrimp will be challenges with the bacterial pathogen and mortality will be compared to control treatment where the animals will be fed pellet without containing any anti-LPS recombinant protein.

REFERENCES

Adams, M. D. *et al.* (1995). Nature 377: 3-17.

Cantor CR (1996) Lighting up hybridization. Nature Biotechnology 14:264-265.

Chang CF, Su MS, Chen HY, Liao IC (2003) Dietary beta-1,3-glucan effectively improves immunity and survival of Penaeus monodon challenged with white spot syndrome virus. Fish Shellfish Immunol 15:297-310.

Chen LL *et al.* (2002) Identification of a nucleocapsid protein (VP35) gene of shrimp white spot syndrome virus and characterization of the motif important for targeting VP35 to the nuclei of transfected insect cells. Virology 293:44-53.

Curtis, M. M., Scharnowske, S., and Watson, A. J. (1992) Sequence and expression of a membrane associated C-type lectin that exhibited CD4-independent binding of human immunodefficiency virus envelope glycoprotein gp120. Proc. Natl. Acad. Sci. 89: 8356-8360.

Dhar AK, Roux MM, Klimpel KR (2001) Detection and quantification of infectious hypodermal and hematopoietic necrosis virus and white spot virus in shrimp using real-time quantitative PCR and SYBR Green chemistry. J Clin Microbiol 39:2835-2845.

Dhar, A. K., Roux, M. M. and Klimpel, K. R. (2002). Quantitative assay for measuring the Taura syndrome virus (TSV) and yellow head virus (YHV) load in shrimp by real-time RT-PCR using SYBR Green chemistry. J. Virol. Methods. 104: 69-82.

Dhar AK, Dettori T, Roux MM, Klimple K, Read B (2003) Identification of differentially expresed genes in white spot syndrome virus infected shrimp (*Penaeus stylirostris*) by cDNA microarrays. Arch Virol 148: 2381-2396.

Dhar, A. K., Cowley, J. A., Hasson, K. W., Walker, P. J., Lightner, D. V. 2004. Genome organization, biology and diagnosis of the Taura syndrome virus (TSV) and yellowhead (YHV) virus of penaeid shrimp. Adv. Virus Res., In Press.

Feinberg, H., Mitchell, D. A., Drickmer, K., and Weis, W. LI. (2001). Structural basis of selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 294: 2163-2166.

Flegel T (1997) Major viral disease of black tiger prawn (*Penaeus monodon*) in Thailand. World J Micro Biotech 13:433-442

Gross, P. S., Barlett, T. C., Browdy, C. L., Chapman, R. W., and Warr, G. W. 2001. Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the Pacific white shrimp, *Litopenaeus vannamei*, and the Atlantic white shrimp, *L. setiferus*. Developmental and Comparative Immunology 25: 565-577.

Inouye K *et al.* (1994) Mass mortality of cultured kuruma shrimp *Penaeus japonicus* in Japan in 1993: Electron microscopic evidence of the causative virus. Fish Pathology 9:149-158

Jory D, Dixon H (1999) Shrimp white spot virus in the Western Hemisphere. Aquacult Mag 25:83-91

Kase, T., Suzuki, Y., Kawai, T., Sakamoto, T., Ohtani, K., Eda, S., Maeda, A., and Okuno, Y. (1999) Human mannan-binding lectin inhibits the infection of influenza A virus without complement. Immunology 97: 385-392.

Kilpatrick, D. C. (2002). Mannan-binding lectin: clinical significance and applications. Biochem. Biophys. Acta 1572: 401-413.

Krishna R, Rao K, Rao PV, Babu P (1997) White spot disease. World Aquaculture 12:14-19

Lehnert, S. A., Wilson, K. J., Byrne, K. and Moore, S. S. 1999. Tissue-specific expressed sequence tags from the black tiger shrimp *Penaeus monodon*. Marine Biotechnology 1: 465-476.

Lightner, D. V. (1996). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA. 304 p.

Lightner, D. V. and Redman, R. M. (1998). Shrimp diseases and current diagnostic methods. Aquaculture 164: 201-220.

Little M, Vonk G (2000) Kit and method for fluorescence based detection assay. In: US Pat. 6,077,669. Becton Dickinson, USA

Liu, Y., Jones, M., Hingtgen, C. M., Bu, G., Laribee, N., Tanzi, R. E., Moir, R. D., Nath, A., and He, J. J. Uptake of HIV-1 Tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. Nature Medicine. 2000. 6:1380-1387.

Livak KJ, Flood SJA, Mermaro J, Mullah KB (2000) Self-quenching fluorescence probe and method. In: Eur Patent Pub 00972848/EP A2. APPLICANT(S)- PERKIN-ELMER CORPORATION (1910480)

Office International des Epizooties (OIE) Fish Disease Commission (2003). Manual of diagnostic tests for aquatic animals. Chapter 4.1.3, White spot disease. <http://www.oie.int/eng/normes/fmanual/A_summry.htm>

Malhotr, R., Haurum, J. S., Thiel, S., Sim, R. B. (1994) Binding of human collectins (SP-A and MBP) to influenja virus. Biochem. J. 304-455-461.

Marks H, Mennens M, Vlak JM, van Hulten MC (2003) Transcriptional analysis of the white spot syndrome virus major virion protein genes. J Gen Virol 84:1517-1523.

Rojtinnakorn, J., Hirono, I., Itami, T., Takahashi, Y., and Aoki, T. Gene expression in haemocytes of kuruma prawn, *Penaeus japonicus*, in response to infection with WSSV by EST approach. Fish and Shellfish Immunology 13: 69-83.

Supungul, P., Klinbunga, S., Pichyangkura, R., Jitrapakdee, S., Hirono, I., Aoki, T., and Tassanakajon, A. 2002. Identification of immune-related genes in hemocytes of black tiger shrimp (*Penaeus monodon)*. Marine Biotechnology 4: 487-494.

Suzuki, T., Yamaya, M., Kamanaka, M., Jia, Y. X., Nakayama, K., Hosoda, M., Yamada, N., Nishimura, H., Sekizawa, K., and Sasaki, H. Type 2 rhinovirus infection of cultured human tracheal epithelial cells: role of LDL recpetor. Am J Physiol Lung Cell Mol Physiol. 2001. 280:L409-L420.

Timmons L, Court DL, Fire A (2001) Ingestion of bacterially expressed dsRNAs

can produce specific and potent genetic interference in *Caenorhabditis elegans*. Gene 263:103-112

Tsai MF *et al.* (2000a) Transcriptional analysis of the ribonucleotide reductase genes of shrimp white spot syndrome virus. Virology 277:92-99.

Tsai MF *et al.* (2000b) Identification and characterization of a shrimp white spot syndrome virus (WSSV) gene that encodes a novel chimeric polypeptide of cellular-type thymidine kinase and thymidylate kinase. Virology 277:100-110.

Tyagi S, Kramer FR (1996) Molecular beacons: Probes that fluoresce upon hybridization. Nature Biotechnology 14:303-308

Tyagi S, Kramer FR, Lizardi PM (2000) Detectably labeled, dual conformation oligo-nucleotide probes, assays and kits. In: US Pat. No. 06103476. PATENT NUMBER- 06103476

van Hulten MC, Goldbach RW, Vlak JM (2000a) Three functionally diverged major structural proteins of white spot syndrome virus evolved by gene duplication. J Gen Virol 81:2525-2529.

van Hulten MC, Westenberg M, Goodall SD, Vlak JM (2000b) Identification of two major virion protein genes of white spot syndrome virus of shrimp. Virology 266:227-236.

van Hulten MC *et al.* (2001) The white spot syndrome virus DNA genome sequence. Virology 286:7-22.

Vasta GR, Quesenberry M, Ahmed H, O'Leary N (1999) C-type lectins and galactins mediate innate and adaptive immune functions: their roles in the complement activation pathway. Dev Comp Immunol 23: 401-420

Vorup-Jensen T, Petersen SV, Hansen AG, Poulsen K, Schwaeble W, Sim RB, Reid KBM, Davis SJ, Thiel S, Jensenius JC (2000) Distinct pathway of mannan-binding lectin (MBL)- and C1-complex autoactivation revealed by reconstitution of MBL with recombinant MBL-associated serine protease-2. J Immunol 165: 2093-2100

Wunschmann, S., Medh, J. D., Klinzmann, D., Warren, S. N., and Stapleton, J. T. Characterization of Hepatitis C Virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. Journal of Virology. 2000. 74:10055-10062.

Yang F *et al.* (2001) Complete genome sequence of the shrimp white spot bacilliform virus. J Virol 75:11811-11820.

Yi G, Qian J, Wang Z, Qi Y (2003) A phage-displayed peptide can inhibit infection by white spot syndrome virus of shrimp. J Gen Virol 84:2545-2553.

Zhang X, Xu X, Hew CL (2001) The structure and function of a gene encoding a basic peptide from prawn white spot syndrome virus. Virus Res 79:137-144.

[0132] The above examples are included for illustrative purposes only and is not intended to limit the scope of the invention. Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

ABSTRACT

The invention pertains to the composition and methods of use of differentially expressed genes in crustaceans, particularly that of shrimp. Genes that have special significance to the ability of shrimp to combat viral disease are described and their selection, amplification, and application for useful purposes. Compositions are described that are useful in feeds, therapeutics, diagnostics, and research. Methods to reduce the effect of viral infection, especially of the white spot syndrome virus, are also described. The uses of the described genes to monitor and diagnose viral disease are also disclosed.

Figure 1. Graphical representation of "Function Classes of genes isolated from hepatopancreas cDNA libraries of healthy and WSSV infected shrimp (*P. vannamei*) that showed similarities with the GenBank database entries.

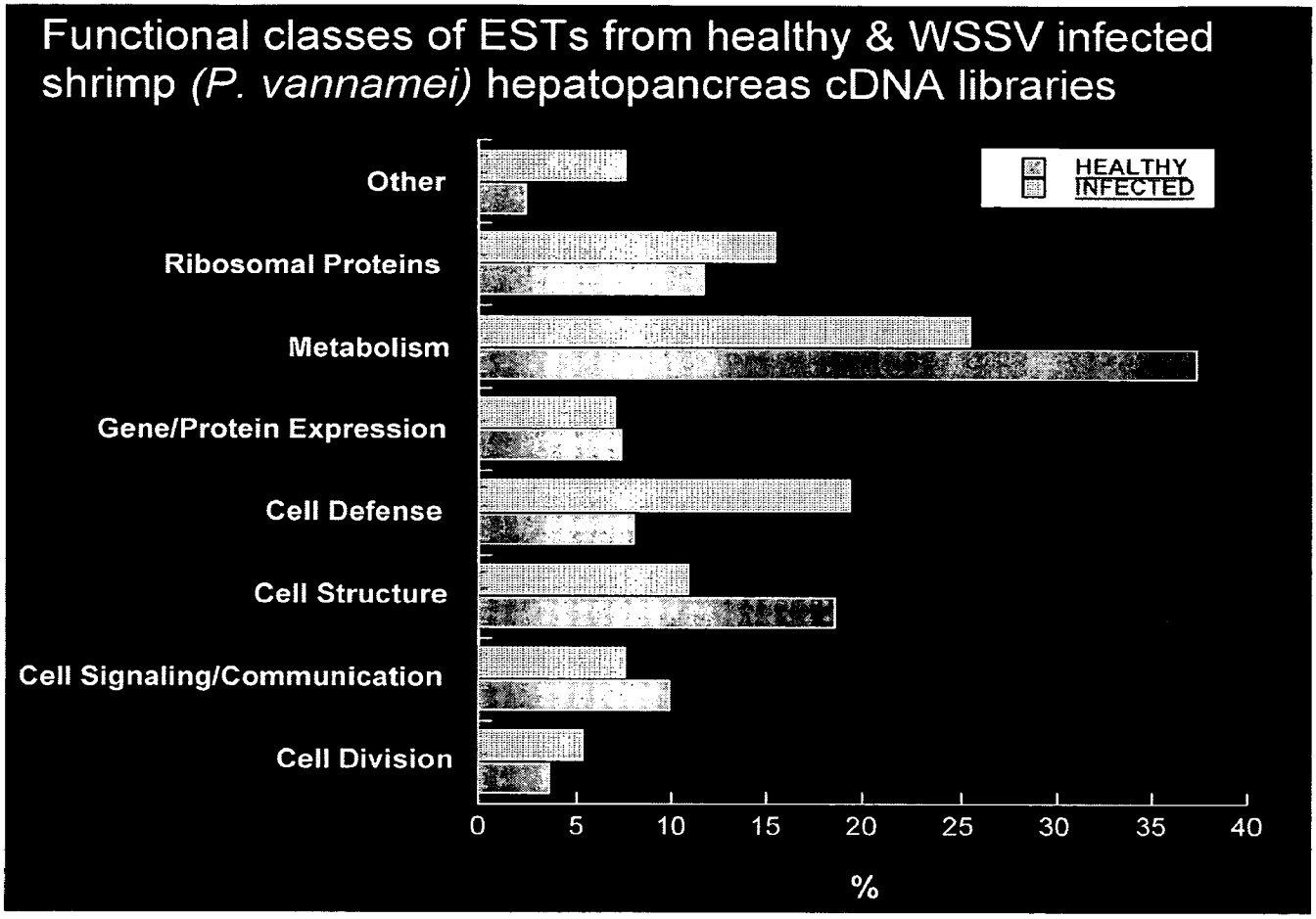
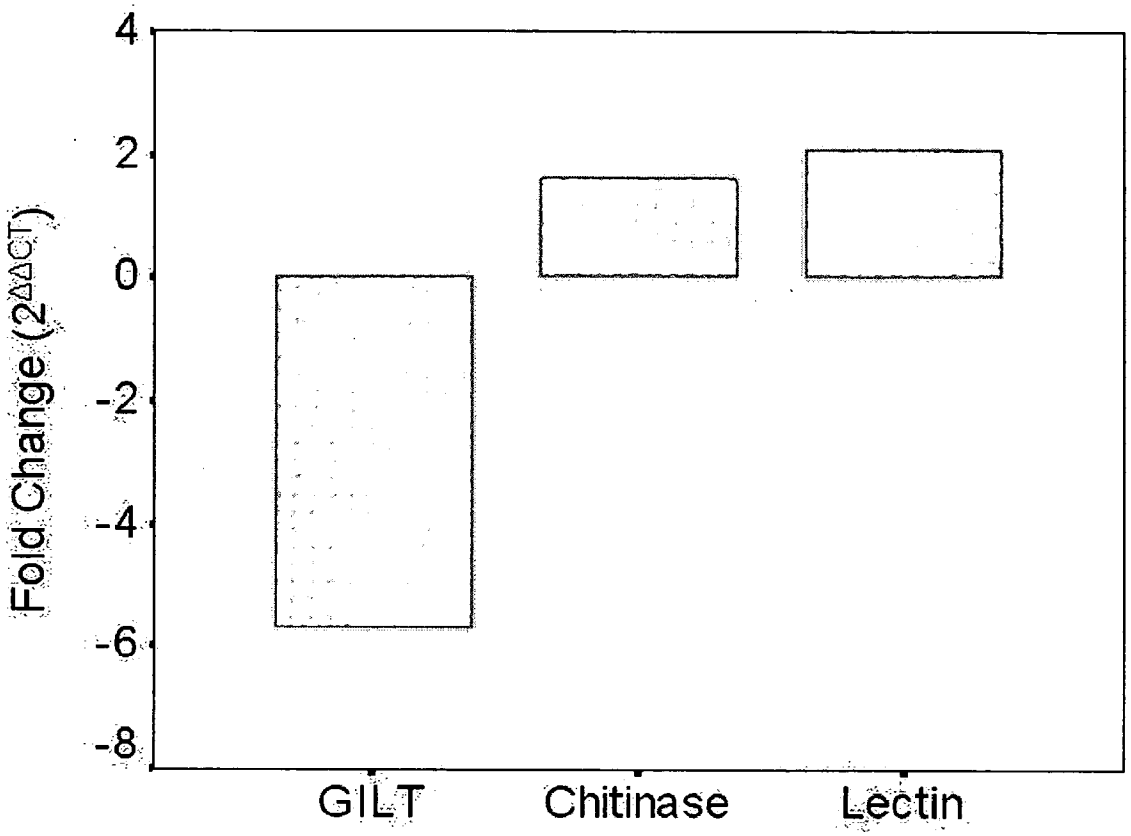


Figure 2. A graphical representation of the differential expression of expressed sequence tags (ESTs) in white spot syndrome virus (WSSV) infected shrimp (*Penaeus vannamei*) compared to healthy shrimp (*Penaeus vannamei*). Bars above the X-axis indicate up-regulation, and bar below the X-axis indicates down-regulation of the gene in WSSV-infected compared to healthy shrimp.



Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Suggested Group Art Unit::	N/A
CD-ROM or CD-R?::	None
Sequence submission?::	Paper
Computer Readable Form (CRF)?::	No
Title::	DISEASE CONTROL IN SHRIMP
Attorney Docket Number::	475443000800
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	2
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	India
Status::	Full Capacity
Given Name::	Arun
Middle Name::	Kumar
Family Name::	DHAR
City of Residence::	San Diego
State or Province of Residence::	CA
Country of Residence::	US
Street of mailing address::	15582 Paseo Ajanta
City of mailing address::	San Diego
State or Province of mailing address::	CA
Postal or Zip Code of mailing address::	92129

Correspondence Information

Correspondence Customer Number:: 25225

Representative Information

Representative Customer Number:: 25225